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(58) Field of Search

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(54) Abstract Title

Human vanilloid receptor-like receptor

(57) A polynucleotide comprising a nucleic acid sequence, preferably that of SEQ ID NO:1, encoding the polypeptide of SEQ ID NO:2, the human vanilloid receptor-like (VR-L) receptor is disclosed. Antisense or complementary sequences to said polynucleotide, antibodies, pharmaceutical and diagnostic compositions related to such polypeptides and polynucleotides are disclosed, as are diagnostic tests for conditions associated with altered VR-L expression and methods of screening for specific binding activity with the VR-L polypeptide.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

INSDOCID: <GB__2346882A_I_>

I F R H I L Q R E F S G L S H L S R K F
ACCGAGTGGTGCTATGGGCCTGTCCGGGTGTCGCTGTATGACCTGGCTTCTGTGGACAGC
5 T E W C Y G P V R V S L Y D L A S V D S
TGTGAGGAGAACTCAGTGCTGGAGATCATTGCCTTTCATTGCAAGAGCCCGCACCGACAC
C E E N S V L E I I A F H C K S P H R H
CGAATGGTCGTTTTTGGAGCCCCTGAACAAACTGCTGCAGGCGAAATGGGATCTGCTCATC
10 R M V V L E P L N K L L Q A K W D L L I
CCCAAGTTCTTCTTAAACTTCCTGTGTAATCTGATCTACATGTTTCATCTTCACCGCTGTT
P K F F L N F L C N L I Y M F I F T A V
GCCTACCATCAGCCTACCCTGAAGAAGCAGGCCGCCCTCACCTGAAAGCGGAGGTTGGA
15 A Y H Q P T L K K Q A A P H L K A E V G
AACTCCATGCTGCTGACGGGGCCACATCCTTATCCTGCTAGGGGGGATCTACCTCCTCGTG
20 N S M L L T G H I L I L L G G I Y L L V
GGCCAGCTGTGGTACTTCTGGCGGCGCCACGTGTTTCATCTGGATCTCGTTCATAGACAGC
G Q L W Y F W R R H V F I W I S F I D S
TACTTTGAAATCCTCTTCCTGTTCCAGGCCCTGCTCACAGTGGTGTCCAGGTGCTGTGT
25 Y F E I L F L F Q A L L T V V S Q V L C
TTCCTGGCCATCGAGTGGTACCTGCCCCTGCTTGTGTCTGCGCTGGTGCTGGGCTGGCTG
F L A I E W Y L P L L V S A L V L G W L
AACCTGCTTTACTATACACGTGGCTTCCAGCACACAGGCATCTACAGTGTCATGATCCAG
30 N L L Y Y T R G F Q H T G I Y S V M I Q
AAGGTCATCCTGCGGGACCTGCTGCGCTTCCTTCTGATCTACTTAGTCTTCCTTTTCGGC
35 K V I L R D L L R F L L I Y L V F L F G
TTCGCTGTAGCCCTGGTGAGCCTGAGCCAGGAGGCTTGGCGCCCCGAAGCTCCTACAGGC
F A V A L V S L S Q E A W R P E A P T G
CCCAATGCCACAGAGTCAGTGCAGCCCATGGAGGGACAGGAGGACGAGGGCAACGGGGCC
40 P N A T E S V Q P M E G Q E D E G N G A
CAGTACAGGGGTATCCTGGAAGCCTCCTTGGAGCTCTTCAAATTCACCATCGGCATGGGC
Q Y R G I L E A S L E L F K F T I G M G
GAGCTGGCCTTCCAGGAGCAGCTGCACTTCCGCGGCATGGTGCTGCTGCTGCTGCTGGCC
45 E L A F Q E Q L H F R G M V L L L L L A
TACGTGCTGCTCACCTACATCCTGCTGCTCAACATGCTCATCGCCCTCATGAGCGAGACC
50 Y V L L T Y I L L L N M L I A L M S E T
GTCAACAGTGTCGCCACTGACAGCTGGAGCATCTGGAAGCTGCAGAAAGCCATCTCTGTC
V N S V A T D S W S I W K L Q K A I S V
CTGGAGATGGAGAATGGCTATTGGTGGTGCAGGAAGAAGCAGCGGGCAGGTGTGATGCTG
55 L E M E N G Y W W C R K K Q R A G V M L
ACCGTTGGCACTAAGCCAGATGGCAGCCCGGATGAGCGCTGGTGCTTCAGGGTGGAGGAG
T V G T K P D G S P D E R W C F R V E E

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GTGAACTGGGCTTCATGGGAGCAGACGCTGCCTACGCTGTGTGAGGACCCGTCAGGGGCA
V N W A S W E Q T L P T L C E D P S G A
5 GGTGTCCCTCGAACTCTCGAGAACCTGTCCTGGCTTCCCCTCCCAAGGAGGATGAGGAT
G V P R T L E N P V L A S P P K E D E D
GGTGCCTCTGAGGAAAACCTATGTGCCCCTCCAGCTCCTCCAGTCCAACTGATGGCCCAGA
G A S E E N Y V P V Q L L Q S N *
10 TGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCAACCACATCTGCTGGCTCTGG
GGTCCCAGT

Figure 2
Effect of Elevated Temperature on Xenopus Oocytes
Expressing the Human VR-L Receptor

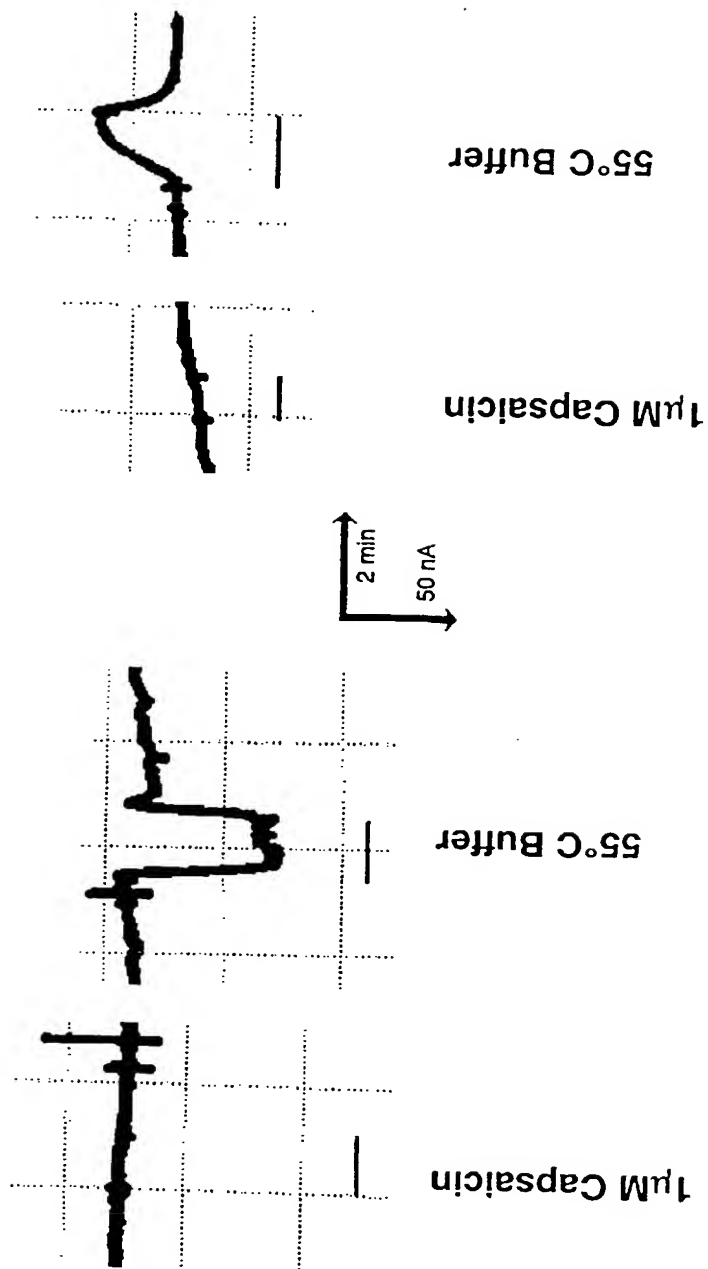


Figure 3:

	104F	CAG	GCC	CGG	GCA	TGC	ACA	TTG
	105T	CCA	GGG	CGA	GGA	CCG	GAA	ATT
5	108F	GAC	AGC	TGG	AGC	ATC	TGG	AAG
	109F	GAC	AGC	TGG	AGC	ATC	TGG	AAG
	110T	CTT	CCA	GAT	GCT	CCA	GCT	GTC
	111T	TTT	GCC	ACC	AGA	ATT	CAC	TGG
	114F	CTC	TCT	TTG	GCC	GCT	TGC	ACC
10	115T	CCA	GCA	CTG	AGT	TCT	CCT	CAC
	118F	GCC	CTA	CCG	TGC	AGC	TTG	AGG
	119T	TGC	CCC	ACG	AGG	AGG	TAG	ATC
	120F	ATG	GCG	ATG	TGC	AGA	GCG	CTG
	121T	AGA	GTC	AAC	CTC	AAA	CTA	CCG
15	126F	GAG	CTT	CTC	CCT	GCG	GTC	AAG
	127T	AAG	GCT	GCT	GAA	AAA	GCA	CTG
	189F	GCT	GGG	CTG	GCT	GAA	CCT	GC
	190T	GAG	GGC	AAT	GAG	CAT	GTT	G

HUMAN VANILLOID RECEPTOR-LIKE RECEPTOR

The present invention is in the field of molecular biology; more particularly, the present invention relates to a nucleic acid sequence and an amino acid sequence for a novel human vanilloid receptor-like receptor (VR-L). The invention further relates to a stable cell line expressing a VR-L receptor and the use of the cell line in a screening technique for the design and development of receptor specific medicaments.

The present invention provides a unique nucleotide sequence which encodes a novel human vanilloid receptor-like (VR-L) receptor. The cDNA, hereinafter designated vr-l, was identified and cloned using IMAGE clones 1286108 and 705190 from human tonsillar cells enriched for germinal centre B cells.

The invention relates to the use of nucleic acid and amino acid sequences of VR-L or its variants, in the diagnosis or treatment of activated, inflamed or diseased cells and/or tissues associated with its expression. Aspects of the invention include the antisense DNA or vr-l; cloning or expression vectors containing vr-l; host cells or organisms transformed with expression vectors containing vr-l; a method for the production and recovery of purified VR-L from host cells; and purified protein, VR-L, which can be used to identify inhibitors for the down regulation of signal transduction involving VR-L.

Figure 1 shows the nucleic acid sequence (coding region of SEQ ID NO: 1) and the predicted amino acid sequence (SEQ ID NO:2) for VR-L.

Figure 2 shows the effects of heat application and 1 μ M capsaicin on oocytes expressing the receptors of the present application.

Figure 3 shows the gene specific primers (SEQ ID NOS:4-19) used to sequence the clones identified as forming part of the VR-L receptor. They are

given as 5' - 3' sequences with the F or T letter denoting whether they were specific for the target sequence in the 5'(F) or 3'(T) direction.

As used herein and designated by the upper case abbreviation, VR-L, refers to a vanilloid receptor-like receptor homologue in either naturally occurring or synthetic form and active fragments thereof which have the amino acid sequence of SEQ ID NO:2. In one embodiment, the polypeptide VR-L is encoded by mRNAs transcribed from the cDNA, as designated by the lower case abbreviation, vr-l, of SEQ ID NO:1.

The novel human vanilloid receptor-like receptor VR-L, which is the subject of this patent application, was discovered among the partial cDNA sequences present in the EST database and the corresponding IMAGE clones.

An "oligonucleotide" is a stretch of nucleotide residues which has a sufficient number of bases to be used as an oligomer, amplimer or probe in a polymerase chain reaction (PCR). Oligonucleotides are usually prepared by chemical synthesis. Their sequence is based on cDNA or genomic sequence information and are used to amplify, reveal or confirm the presence of a similar DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 35 nucleotides, preferably about 25 nucleotides.

"Probes" may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or be chemically synthesised. They are useful in detecting the presence of identical or similar sequences.

A "portion" or "fragment" of a polynucleotide or nucleic acid comprises all or part of the nucleotide sequence having fewer nucleotides than about 6 kb, preferably fewer than about 1 kb which can be used as a probe. Such probes may be labelled with reporter molecules using nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. After pre-testing to optimise reaction conditions and to eliminate false positives,

nucleic acid probes may be used in Southern, Northern or in situ hybridizations to determine whether DNA or RNA encoding VR-L is present in a cell type, tissue, or organ.

5 “Reporter” molecules are those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents which associate with, establish the presence of, and may allow quantification of a particular nucleotide or amino acid sequence.

10 “Recombinant nucleotide variants” encoding VR-L may be synthesised by making use of the “redundancy” in the genetic code. Various codon substitutions, such as the silent changes which produce specific restriction sites or codon usage-specific mutations, may be introduced to optimise cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic host system, respectively.

15 “Chimeric” molecules may be constructed by introducing all or part of the nucleotide sequence of this invention into a vector containing additional nucleic acid sequence which might be expected to change any one (or more than one) of the following VR-L characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signalling, etc.

20 “Active” refers to those forms, fragments, or domains of any VR-L polypeptide which retain the biological and/or antigenic activities of any naturally occurring VR-L.

25 “Naturally occurring VR-L” refers to a polypeptide produced by cells which have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

 “Derivative” refers to those polypeptides which have been chemically modified by such techniques as ubiquitination, labelling (see above),

pegylation (derivatization with polyethylene glycol), and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins.

5 “Recombinant polypeptide variant” refers to any polypeptide which differs from naturally occurring VR-L by amino acid insertions, deletions and/or substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities or interest may be found by comparing the sequence of VR-L with that of related polypeptides and minimizing the
10 number of amino acid sequence changes made in highly conserved regions.

Amino acid “substitutions” are conservative in nature when they result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

15 “Insertions” or “deletions” are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by producing the peptide synthetically or by systematically making insertions, deletions, or substitutions of nucleotides in the vr-l sequence using recombinant DNA techniques.

20 An “oligopeptide” is a short stretch of amino acid residues and may be expressed from an oligonucleotide. It may be functionally equivalent to and the same length as (or considerably shorter than) a “fragment”, “portion”, or “segment” of a polypeptide. Such sequences comprise a stretch of amino acid residues of at least about 5 amino acids and often about 17 or more amino
25 acids, typically at least about 9 to 13 amino acids, and of sufficient length to display biological and/or antigenic activity.

“Inhibitor” is any substance which retards or prevents a chemical or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, and antagonists.

“Standard” expression is a quantitative or qualitative measurement for comparison. It is based on a statistically appropriate number of normal samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles.

The present invention provides a nucleotide sequence uniquely identifying a novel human vanilloid receptor-like receptor. Because the receptor is responsive to heat, the nucleic acids (vr-l), polypeptides (VR-L) and antibodies to VR-L are useful in diagnostic assays which survey for increased receptor production or function. A diagnostic test for excessive expression of VR-L can accelerate diagnosis and proper treatment of abnormal conditions associated with pain.

The nucleotide sequences encoding VR-L (or their complement) have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of VR-L, and use in generation of antisense DNA or RNA, their chemical analogues and the like. Uses of nucleotides encoding VR-L disclosed herein are exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, e.g. the triplet genetic code, specific base pair interactions, etc.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of VR-L-encoding nucleotide sequences may be produced. Some of these will only bear minimal homology to the nucleotide sequence of the known and naturally occurring VR-L. The

invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of
5 naturally occurring vr-l, and all such variations are to be considered as being specifically disclosed. The variant given in Figure 1 is preferred.

Although the nucleotide sequences which encode VR-L, its derivatives or its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring vr-l under stringent conditions, it may be
10 advantageous to produce nucleotide sequences encoding VR-L or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other
15 reasons for substantially altering the nucleotide sequence encoding VR-L and/or its derivatives without altering the encoded aa sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

20 Nucleotide sequences encoding VR-L may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA-techniques (Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons,
25 New York City). Useful nucleotide sequences for joining to vr-l include an assortment of cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and the like. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, etc. In general, vectors of interest may contain an origin of replication functional

in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for one or more host cell systems.

Another aspect of the subject invention is to provide for vr-l-specific hybridization probes capable of hybridizing with naturally occurring
5 nucleotide sequences encoding VR-L. Such probes may also be used for the detection of similar sequences and should preferably contain at least 50% of the nucleotides from the vr-l sequence. The hybridization probes of the present invention may be derived from the nucleotide sequence presented as SEQ ID NO:1 or from genomic sequences including promoters, enhancers or
10 introns of the native gene. Hybridization probes may be labeled by a variety of reporter molecules using techniques well known in the art.

PCR as described US Patent Nos. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes VR-L. Such probes used in PCR may be of
15 recombinant origin, chemically synthesised, or a mixture of both. Oligomers may comprise discrete nucleotide sequences employed under optimised conditions for identification of vr-l in specific tissues or diagnostic use. The same two oligomers, a nested set of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for identification
20 of closely related DNAs or RNAs.

Other means of producing specific hybridization probes for vr-l include the cloning of nucleic acid sequences encoding VR-L or VR-L derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available and may be used to synthesise RNA probes in
25 vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate reporter molecules.

It is possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. After synthesis, the nucleic acid sequence can be inserted into any of the many available DNA vectors and their respective host

cells using techniques which are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into the nucleotide sequence. Alternatively, a portion of sequence in which a mutation is desired can be synthesised and recombined with longer portion of an existing genomic or
5 recombinant sequence.

The nucleotide sequence for vr-l can be used in an assay to detect inflammation or disease associated with abnormal levels of VR-L expression. The cDNA can be labeled by methods known in the art, added to a fluid, cell or tissue sample from a patient, and incubated under hybridising conditions.
10 After an incubation period, the sample is washed with a compatible fluid which contains a reporter molecule. After the compatible fluid is rinsed off, the reporter molecule is quantitated and compared with a standard as previously defined. If kinase expression is significantly different from standard expression, the assay indicates inflammation or disease.

15 The nucleotide sequence for vr-l can be used to construct hybridisation probes for mapping the native gene. The gene may be mapped to a particular chromosome or to a specific region of a chromosome using well known mapping techniques. These techniques include in situ hybridisation of chromosomal spreads (Verma et al (1988) Human Chromosomes: A Manual
20 of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries.

In situ hybridisation of chromosomal preparations and physical
25 mapping techniques such a linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of genetic map data can be found in the yearly genome issue of Science (eg 1994, 265:1981f). Often locating a gene on the chromosome of another mammalian species may

reveal associated markers which can be used to help identify the analogous human chromosome.

New nucleotide sequences can be assigned to chromosomal subregions by physical mapping. The mapping of new genes or nucleotide sequences provide useful landmarks for investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT) has been crudely localised by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent or reveal genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in gene sequence between normal and carrier or affected individuals.

Nucleotide sequences encoding vr-l may be used to produce a purified oligo- or polypeptide using well known methods of recombinant DNA technology. Goeddel (1990, Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego CA) is one among many publications which teach expression of an isolated nucleotide sequence. The oligopeptide may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which the nucleotide sequence was derived or from a different species. Advantages of producing an oligonucleotide by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Cells transformed with DNA encoding VR-L may be cultured under conditions suitable for expression of their extracellular, transmembrane or intracellular domains and recovery of such peptides from cell culture. VR-L (or any of its domains) produced by a recombinant cell may be secreted or may be contained intracellularly, depending on the particular genetic construction used. In general, it is more convenient to prepare recombinant

proteins in secreted form. Purification steps vary with the production process and the particular protein produced. Often an oligopeptide can be produced from a chimeric nucleotide sequence. This is accomplished by ligating the nucleotides from vr-l or a desired portion of the polypeptide to a nucleic acid sequence encoding a polypeptide domain which will facilitate protein purification (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

In addition to recombinant production, fragments of VR-L may be produced by direct peptide synthesis using solid-phase techniques (eg Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco CA; Merrifield J (1963) J Am Chem Soc 85:2149-2154). Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, CA) in accordance with the instructions provided by the manufacturer. Additionally, a particular portion of VR-L may be mutated during direct synthesis and combined with other parts of the peptide using chemical methods.

VR-L for antibody induction does not require biological activity; however, the protein must be antigenic. Peptides used to induce specific antibodies may have an aa sequence consisting of at least five aa, preferably at least 10 aa. They should mimic a portion of the aa sequence of the protein and may contain the entire aa sequence of a small naturally occurring molecule such as VR-L. An antigen portion of VR-L may be fused to another protein such as keyhole limpet hemocyanin, and the chimeric molecule used for antibody production.

Antibodies specific for VR-L may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. An antibody is specific for VR-L if it is produced against an epitope of the polypeptide and binds to a least part of the natural or recombinant protein. Antibody production includes not only the stimulation of an immune response by injection into animals, but also analogous processes such as the production

of synthetic antibodies, the screening of recombinant immunoglobulin libraries for specific-binding molecules (eg Orlandi R et al (1989) PNAS 86:3833-3837, or Huse WD et al (1989) Science 256:1275-1281) or the in vitro stimulation of lymphocyte populations. Current technology (Winter G and
5 Milstein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules which specifically bind VR-L.

An additional embodiment of the subject invention is the use of VR-L
10 specific antibodies, inhibitors or receptors as bioactive agents to treat pain.

Bioactive compositions comprising agonists, antagonists, receptors or inhibitors of VR-L may be administered in a suitable therapeutic dose determined by any of several methodologies including clinical studies on mammalian species to determine maximal tolerable dose and on normal
15 human subjects to determine safe dose. Additionally, the bioactive agent may be complexed with a variety of well established compounds or compositions which enhance stability or pharmacological properties such as half-life. It is contemplated that the therapeutic, bioactive composition may be delivered by intravenous infusion into the bloodstream or any other effective means which
20 could be used for treating problems involving excessive lymphocyte and leukocyte trafficking.

The examples below are provided to illustrate the subject invention.

IDENTIFICATION OF VR-L RECEPTOR

25 The vr-l sequence of the present invention was identified in the partial sequences of two IMAGE clones (1286108 and 705190) among the sequences comprising a human tonsillar cell library enriched for germinal centre B cells. The enrichment was carried out by flow sorting (CD20+, IgD-). cDNA synthesis was primed with a NotI – oligo (dT) primer

[5'-TGTTACCAATCTGAAGTGGGAGCGGCCGCCTCATTTTTTTTTTTTTTTT
TTT-3'] (SEQ. ID. NO:3). Double stranded cDNA was ligated to Eco RI
adaptors (Pharmacia), digested with NotI and cloned into the NotI and EcoRI
sites of the modified pT7T3 vector. The library went through one round of
5 normalisation.

An EST database was searched using the blast (versions 1.4 and 2.0),
fasta and Smith-Waterman algorithms with the rat VR1 DNA or protein
sequence, and a number of human sequences with partial homology to VR1
were obtained. The cDNA clones used to derive these EST sequences were
10 obtained from the IMAGE consortium and sequenced using the gene specific
primers shown in Figure 3 (SEQ ID NOS:4-19).

Table I shows, by Genbank accession number, the identity of a number
of human sequences with partial homology to VR1 thus identified.

15 Table 1:

H99578	N24224	N21167	AA815110
N21284	AA357145	AA814328	H50364
AA281348	W38665	H27879	AA461295
N35179	N23395	H49060	H51393
AA304033	W44731	H21490	H20025
T12251	AA281349	AA236417	AA768829
W92895	AA236416	N26729	AA741232

Blast, which stands for Basic Local Alignment Search Tool, was used to
search for local sequence alignments (Altschul et al (1997) Nucleic Acids Res.
25:3389-3402, Altschul et al (1993) J Mol Evol 36:290-300; Altschul, SF et al
20 (1990) J Mol Biol 215:403-10). BLAST produces alignments of both
nucleotide and aa sequences to determine sequence similarity. Because of the
local nature of the alignments, blast is especially useful in determining exact
matches or in identifying homologs. Whereas it is ideal for matches which do
not contain gaps, it is inappropriate for performing motif-style searching.

The fundamental unit of blast algorithm output is the High-scoring Segment Pair (HSP).

5 An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The blast approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant
10 threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

15 Two cDNA clones (IMAGE clones 1286108 and 705190) were identified as together containing the entire coding region of the VR-L gene. DNA from these clones was digested with EcoRI (New England Biolabs) and the ends blunted by treatment with DNA Polymerase I (Klenow-Fragment, New England Biolabs) and dephosphorylated by treatment with Alkaline Phosphatase (calf-intestinal, Boehringer). The DNA was further digested
20 with NotI (New England Biolabs). The resulting ~ 680 bp fragment from clone 1286108 and the ~ 1791 bp fragment from clone 705190 were purified and ligated using T4 DNA ligase. Ligation products corresponding to the ligation of 5'-1286108-3' to 5'-705190-3' (2471 bp) were purified and ligated into the mammalian expression vector pcDNA3.1Zeo+ (Invitrogen).
25 Resultant clones were screened by restriction digest, and the insert completely sequenced on both strands (Figure 1).

The tissue expression of the vr-l mRNA was determined by radioactive hybridisation of a random primed probe made from a 1346 bp region of the human vr-l cDNA (bases 735 to 2081) against poly (A)+ RNA isolated from a

variety of human tissues, and supplied by Clontech (Human MasterBlot). The highest levels of specific hybridisation were found in lung, spleen, small intestine, thymus, placenta, and lymph node.

5 FUNCTIONAL CHARACTERISATION OF THE *vr-1* GENE
 PRODUCT

Methods

Xenopus oocyte expression

 Adult female *Xenopus laevis* were anaesthetized by immersion in a
10 0.4% solution of 3-aminobenzoic acid ethylester for 30-45 mins (or until
 unresponsive). Ovary tissue was removed via a small abdominal incision and
 Stage V and VI oocytes were isolated with fine forceps. After mild collagenase
 treatment to remove follicle cells (Type 1A, 0.5 mg mL⁻¹, for 8 mins), the
 oocyte nuclei were directly injected with 10-20 nl of injection buffer (88 mM
15 NaCl, 1 mM KCl, 15 mM HEPES, at pH 7, filtered through nitrocellulose) or
 sterile water containing *vr-1* cDNA engineered into the expression vector
 pcDNA3.1Zeo. Following incubation for 48 hrs, oocytes were placed in a 50 µL
 bath and perfused at 4-6 mL min⁻¹ with modified Barth's medium (MBS)
 consisting of 88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM MgSO₄, 0.33
20 mM Ca(NO₃)₂, 0.91 mM CaCl₂, 2.4 mM NaHCO₃, at pH 7.5. Cells were
 impaled with two 1-3 MΩ electrodes containing 2 M KCl and voltage clamped
 between at -70mV. Drugs were applied in the perfusate and heat was applied
 by bath applying preheated MBS.

Whole-cell patch clamp

25 A glass coverslip containing a monolayer of human embryonic kidney
 (HEK) 293 cells transiently transfected with the *vr-1* cDNA was placed in a
 perspex chamber mounted on the stage of a Nikon Diaphot inverted phase-
 contrast microscope and continuously perfused with calcium-free artificial
 cerebrospinal fluid (aCSF, see below). Fire-polished patch pipettes were

pulled on program 10 of a WZ, DMZ-Universal puller using conventional 120TF-10 electrode-glass. Pipette tip diameter was generally 1.5-2.5 μ M, resistances were approximately 4M Ω . The intracellular pipette solution used is detailed below. High resistance (>10 G Ω) seals were formed by advancing
5 the pipette onto the cell and exerting gentle suction to break through the membrane. The cells were voltage-clamped at -60mV, using an Axon Axopatch 2 amplifier. Drugs or heated aCSF were applied to the cell under study by fast perfusion using a large internal diameter (50-100 μ M) triple-barrel pipette assembly. Drug responses were obtained by rapidly positioning
10 the perfusion pipette to completely envelop the cell in drug solution. This was achieved via a Biologic rapid solution changer which pivots the barrels into the desired position. Fast washout was obtained by re-positioning the washout barrel in line with the cell.

15 Composition of calcium-free artificial cerebrospinal fluid (aCSF).

	NaCl	149mM
	KCl	3.25mM
	MgCl ₂	2mM
20	HEPES	10mM
	D-Glucose	11mM
	D(+)-Sucrose	22mM
	pH 7.4 with NaOH	

Composition of caesium chloride intracellular pipette solution.

	Salt	Stock(M)	(mM)	ml/20ml
5	CsCl	1	130	2.6
	HEPES	1	10	0.2
	BAPTA.Cs		10	200mg
	ATP.Mg		5	50.72
	Leupeptin		0.1	0.93mg
10	MgCl ₂	1	1	20μl

pH = 7.3 320 - 340mOsm

Results

15 Human vr-l cDNA was expressed in *Xenopus* oocytes and in HEK 293 cells. In both expression systems inward currents were recorded to application of buffer warmed to 55°C, applied directly to the cell or via the bathing medium, which were not present in untransfected HEK cells or uninjected oocytes. No responses were produced by application of 1μM
20 capsaicin. Figure 2 shows the effect of heat application and 1μM capsaicin in (a) VR-L expressing oocytes and (b) control oocytes.

 Knowledge of the correct, complete cDNA sequence of VR-L enables its use as a tool for antisense technology in the investigation of gene function. Oligonucleotides, cDNA or genomic fragments comprising the antisense
25 strand of vr-l are used either in vitro or in vivo to inhibit expression of the mRNA. Such technology is now well known in the art, and antisense molecules can be designed at various locations along the nucleotide sequences. By treatment of cells or whole test animals with such antisense sequences, the gene of interest is effectively turned off. Frequently, the

function of the gene is ascertained by observing behavior at the intracellular, cellular, tissue or organismal level (eg. lethality, loss of differentiated function, changes in morphology, etc.)

5 In addition to using sequences constructed to interrupt transcription of a particular open reading frame, modifications of gene expression are obtained by designing antisense sequences to intron regions, promoter/enhancer elements, or even to transacting regulatory genes. Similarly, inhibition is achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing.

10 Two approaches are utilised to raise antibodies to VR-L and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from reverse phase HPLC separation is obtained in quantities up to 75mg. This denature protein is used to immunise mice or rabbits using standard protocols; about 100 µg are
15 adequate for immunisation of a mouse, while up to 1 mg might be used to immunise a rabbit. For identifying mouse hybridomas, the denatured protein is radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labelling and screening of several
20 thousand clones.

In the second approach, the amino acid sequence of an appropriate VR-L domain, as deduced from translation of the cDNA, is analysed to determine regions of high antigenicity. Oligopeptides comprising appropriate hydrophilic regions are synthesised and used in suitable immunisation
25 protocols to raise antibodies. Analysis to select appropriate epitopes is described by Ausubel FM et al (supra). The optimal amino acid sequences for immunisation are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be

exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesised using an Applied Biosystems Peptide Synthesiser Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH; 5 Sigma, St Louis MO) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine is introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunised with the peptide-KLH complex in complete 10 Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% bovine serum albumin, reacting with antisera, washing and reacting with labelled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. 15 Hybridomas of interest are detected by screening with labelled VR-L to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto CA) are coated during incubation with affinity purified, specific rabbit anti-mouse (or suitable antispecies Ig) antibodies at 10 mg/ml. The 20 coated wells are blocked with 1% BSA, washed and incubated with supernatants from hybridomas. After washing the wells are incubated with labelled VR-L at 1 mg/ml. Supernatants with specific antibodies bind more labelled VR-L than is detectable in the background. Then clones producing specific antibodies are expanded and subjected to two cycles of cloning at 25 limiting dilution. Cloned hybridomas are grown in tissue culture by standard methods. Monoclonal antibodies with affinities of at least 10^8 M^{-1} , preferably 10^9 to 10^{10} or stronger, are typically made by standard procedure as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and in Goding (1986)

Monoclonal Antibodies: Principles and Practice, Academic Press, New York City.

Particular VR-L antibodies are useful for investigating signal transduction and the diagnosis of infectious or hereditary conditions which
5 are characterised by differences in the amount or distribution of VR-L or downstream products of an active signalling cascade.

Diagnostic tests for VR-L include methods utilising an antibody and a label to detect VR-L in human body fluids, membranes, cells, tissues or extracts of such. The polypeptides and antibodies of the present invention
10 are used with or without modification. Frequently, the polypeptides and antibodies are labelled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels
15 include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like. Patents teaching the use of such labels include US Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in
20 US Patent No. 4,816,567.

A variety of protocols for measuring soluble or membrane-bound VR-L, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting
25 (FACS). A two-site monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes on VR-L is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211f).

Native or recombinant VR-L is purified by immunoaffinity chromatography using antibodies specific for VR-L. In general, an immunoaffinity column is constructed by covalently coupling the anti-TRH antibody to an activated chromatographic resin.

5 Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway NJ). Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is
10 coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

A soluble VR-L containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of VR-L (eg. high ionic strength buffers in
15 the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/protein binding (eg. a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and VR-L is collected.

This invention is particularly useful for screening therapeutic
20 compounds by using VR-L or binding fragments thereof in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test is either free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilises eukaryotic or prokaryotic host cells which are stably transformed with recombinant
25 nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, are used for standard binding assays. One measures, for example, the formation of complexes between VR-L and the

agent being tested. Alternatively, one examines the diminution in complex formation between VR-L and a receptor caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which affect signal transduction. These methods, well known in the art, comprise contacting such a agent with VR-L polypeptide or a fragment thereof and assaying (i) for the presence of a complex between the agent and the VR-L polypeptide or fragment, or (ii) for the presence of a complex between the VR-L polypeptide or fragment and the cell. In such competitive binding assays, the VR-L polypeptide or fragment is typically labelled. After suitable incubation, free VR-L polypeptide or fragment is separated from the present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to VR-L or to interfere with the VR-L and agent complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the VR-L polypeptides and is described in detail in European Patent Application 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesised on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with VR-L polypeptide and washed. Bound VR-L polypeptide is then detected by methods well known in the art. Purified VR-L are also coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralising antibodies are used to capture the peptide and immobilise it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralising antibodies capable of binding VR-L specifically compete with a test compound for binding to VR-L polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic determinants with VR-L.

The goal of rational drug design is to produce structural analogues of biologically active polypeptides of interest or of small molecules with which they interact, agonists, antagonists, or inhibitors. Any of these examples are used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo (eg. 5 Hodgson J (1991) Bio/Technology 9:19-21).

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor complex, is determined by x-ray crystallography, by computer modelling or, most typically, by a combination 10 of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide is gained by modelling based on the structure of homologous proteins. In both cases, relevant structural information is used to design 15 efficient inhibitors. Useful examples of rational drug design includes molecules which have improved activity or stability as shown by Braxton S and Wells JA (1992, Biochemistry 31:7796-7801) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda SB et al (1993 J Biochem 113:742-46), incorporated herein by reference.

20 It is possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design is based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, 25 pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids is expected to be an analogue of the original receptor. The anti-id is then used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides then act as the pharmacore.

By virtue of the present invention, sufficient amount of polypeptide are made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the VR-L amino acid sequence provided herein provides guidance to those employing computer modelling techniques in place
5 of or in addition to x-ray crystallography.

The inventive purified VR-L is a research tool for identification, characterisation and purification of interacting G or other signal transduction pathway proteins. Radioactive labels are incorporated into a selected VR-L domain by various methods known in the art and used in vitro to capture
10 interacting molecules. A preferred method involves labelling the primary amino groups in VR-L with ^{125}I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J 133: 529). This reagent has been used to label various molecules without concomitant loss of biological activity (Hebert CA et al (1991) J Biol Chem 266: 18989; McColl S et al (1993) J Immunol
15 150:4550-4555).

Labeled VR-L is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, membrane-bound VR-L is covalently coupled to a chromatography column. Cell-free extract derived from synovial cells or putative target cells is passed
20 over the column, and molecules with appropriate affinity bind to VR-L. VR-L-complex is recovered from the column, and the VR-L-binding ligand disassociated and subjected to N-terminal protein sequencing. This aa sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an
25 appropriate cDNA library.

In an alternate method, antibodies are raised against VR-L, specifically monoclonal antibodies. The monoclonal antibodies are screened to identify those which inhibit the binding of labelled VR-L. These monoclonal antibodies are then used therapeutically.

Antibodies, inhibitors, or antagonists of VR-L (or other treatments to limit signal transduction, LST) provide different effects when administered therapeutically. LSTs are formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although pH may vary according to the characteristics of the antibody, inhibitor, or antagonist being formulated and the condition to be treated. Characteristics of LSTs include solubility of the molecule, half-life and antigenicity/immunogenicity. These and other characteristics aid in defining an effective carrier. Native human proteins are preferred as LSTs, but organic or synthetic molecules resulting from drug screens are equally effective in particular situations.

LSTs are delivered by known routes of administration including but not limited to topical creams and gels; transmucosal sprays and aerosols; transdermal patches and bandages; injectable, intravenous and lavage formulations; and orally administered liquids and pills particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration is determined by the attending physician and varies according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the LST to be administered, and the pharmacokinetic profile of a particular LST. Additional factors which are taken into account include severity of the disease state, patient's age, weight, gender and diet, time and frequency of LST administration, possible combination with other drugs, reaction sensitivities, and tolerance/response to therapy. Long acting LST formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular LST.

Normal dosage amounts vary from 0.1 to 100,000 μg , up to a total dose of about 1 g, depending upon the route of administration. Guidance as to

particular dosages and methods of delivery is provided in the literature; see
US Patent Nos. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art
employ different formulations for different LSTs. Administration to cells
such as nerve cells necessitates delivery in a manner different from that to
5 other cells such as vascular endothelial cells.

It is contemplated that abnormal signal transduction, trauma, or
diseases which trigger VR-L activity are treatable with LSTs. These
conditions or diseases are specifically diagnosed by the tests discussed above,
and such testing should be performed in suspected cases of viral, bacterial or
10 fungal infections; allergic responses; mechanical injury associated with
trauma; hereditary diseases; lymphoma or carcinoma; or other conditions
which activate the genes of lymphoid tissues.

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide of SEQ ID NO:2, or the complement of said polynucleotide.
- 5 2. The polynucleotide of Claim 1 comprising the nucleic acid sequence of SEQ ID NO:1.
3. An antisense molecule comprising the complement of the polynucleotide of Claim 2 or a portion thereof.
4. A pharmaceutical composition comprising the antisense molecule of
10 Claim 3 and a pharmaceutically acceptable excipient.
5. A diagnostic composition comprising an oligomer of the polynucleotide of Claim 2.
6. A diagnostic test for a condition associated with altered VR-L expression comprising the steps of:
15 a) providing a biological sample;
 b) combining the biological sample and the diagnostic composition of Claim 5;
 c) allowing hybridisation to occur between the biological sample and the diagnostic composition under suitable conditions;
20 d) measuring the amount of hybridisation to obtain a sample value; and
 e) comparing the sample value with standard values to determine whether vr-l expression is altered.
7. An expression vector comprising the polynucleotide of Claim 1.
- 25 8. A host cell transformed with the expression vector of Claim 7.
9. A method of producing a polypeptide, said method comprising the steps of:
 a) culturing the host cell of Claim 8 under conditions suitable for the expression of the polypeptide; and

- b) recovering the polypeptide from the host cell culture.
- 10. A purified polypeptide (VR-L) comprising the amino acid sequence of SEQ ID NO:2.
- 11. A diagnostic composition comprising the polypeptide of Claim 10 or a portion thereof.
- 12. A pharmaceutical composition comprising the polypeptide of Claim 10 and a pharmaceutically acceptable excipient.
- 13. An antibody specific for the purified polypeptide of Claim 9, or for a portion of that polypeptide.
- 10 14. A diagnostic composition comprising the antibody of Claim 13.
- 15. A diagnostic test for a condition associated with altered VR-L expression comprising the steps of:
 - a) providing a biological sample;
 - b) combining the biological sample and the antibody of Claim 13 under conditions suitable for complex formation;
 - c) measuring the amount of complex formation between VR-L and the antibody to obtain a sample amount; and
 - d) comparing the amount of complex formation in the sample with standard amounts of complex formation, wherein a variation between sample amount and standard amounts of complex formation establishes the presence of the condition.
- 16. A method of screening a plurality of compounds for specific binding affinity with the polypeptide of Claim 10 or any portion thereof comprising the steps of:
 - a) providing a plurality of compounds;
 - b) combining VR-L with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and
 - c) detecting binding of VR-L to each of the plurality of compounds, thereby identifying the compounds which specifically bind VR-L.

17. A pharmaceutical composition comprising a compound of Claim 16 and a pharmaceutically acceptable excipient.



INVESTOR IN PEOPLE

Application No: GB 9928566.0
Claims searched: 1-17

Examiner: Dr Patrick Purcell
Date of search: 6 June 2000

Patents Act 1977 Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:
UK Cl (Ed.R):
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Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
P, X	EP 0953638 A1 (SYNTHELABO) see whole document, especially claims and SEQ ID NOS:1 & 2	1-17
E, X	WO 00/22121 A2 (UNI. COLLEGE LONDON) see whole document, especially page 7, lines 5-19, page 8, lines 27-30, page 17, lines 27-31, page 19, lines 21-22, page 24, lines 3-6, page 25, line 15-page 26, line 2 and Figs. 3a & 3b	1-17
P, X	WO 99/37765 A1 (SMITHKLINE BEECHAM PLC), see whole document, especially page 2, lines 2-7, page 3, lines 10-18, page 7, lines 18-24, page 8, line 33-page 9, line 34, page 11, lines 5-9, page 12, line 3-page 13, line 31 and SEQ ID NOS: 1, 2, 5 & 6	1-17
P, X	WO 99/37675 A1 (THE REGENTS OF THE UNI. OF CALIFORNIA) see whole document, especially page 2, line 35-page 3, line 29, page 16, lines 9-13, page 21, lines 26-36, page 23, lines 27-33, page 25, lines 18-22, page 30, lines 8-10 and SEQ ID NOS: 35 & 36	1-17

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.



INVESTOR IN PEOPLE

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